Influenza A virus infection of pigeons

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Influenza A viruses of all subtypes, in their low pathogenic state, form part of the natural microbiota of water bird species. The viruses multiply in the epithelial cells lining respiratory system and the gut to shed in oral secretions and faeces into the environment, without the host showing any clinical signs. The viral subtypes (or serotypes) are designated by the combination of two major antigens projecting from surface of the virus, namely the hemagglutin (H; H1 to H16) and neuraminidase (N; N1 to N9) proteins, e.g. H5N1. Of all the influenza A subtypes, only H5Nx and H7Nx viruses (where x denotes any N-type) are cause the highly-contagious and lethal disease of poultry and humans known as avian influenza. When low pathogenic viruses (LPAI) H5Nx or H7Nx viruses circulate for extended periods in susceptible poultry flocks, the highly pathogenic forms (HPAI) can arise through mutational events in the viral RNA genome, but especially in the gene encoding the hemagglutinin protein. Whereas LPAI viruses are localised in their infection of epithelial cells and cause mild respiratory infections, conjunctivitis and drops in egg production from which poultry usually recover, HPAI viruses are able to invade a much wider range of cell and tissue types, causing a systemic infection that typically manifests as depression with ruffled feathers, conjunctivitis, cyanosis of the unfeathered skin, severe respiratory and/or neurological signs followed by rapid death.

The pathogenic potential of H5Nx and H7Nx LPAI viruses is the reason for their notifiable status, as the conversion of LPAI to HPAI H5Ns or H7Nx in poultry has been documented on 42 occasions since 1959. Forty of these mutational events resulted in highly localised, single-country epidemics, most of which endured for less than a year, with few lasting up to two years. The exceptions were the continuing outbreaks of H7N3 in Mexico that started in 2012, and the H7N9 outbreaks in China from 2016 to 2019. Only two of the forty two HPAI mutation events involved the spread of the virus to multiple countries. In 2003, an H7N7 HPAI epidemic affected poultry of the Netherlands, Belgium and Germany, but

was eventually brought under control by strict measures that included movement restrictions and the mass culling of infected flocks.

The second HPAI mutational event that affected multiple countries is unprecedented in the scale and magnitude of the outbreaks it caused across the globe. In 1996 an H5N1 HPAI virus emerged from the live bird markets in China, and its descendants became known as the Goose/Guangdong H5N1 lineage. As time progressed, clades and sub-clades of the virus diverged and eventually the poultry-adapted strains spilled back to wild birds and became established in the intercontinental migration flyways that stretch from South-East Asia to Siberia, across to Western Europe, the Middle East and south as far as West and Central Africa.

Four intercontinental transmission waves of Goose/Guangdong H5 lineage viruses swept the globe since 2005, each characterized by epidemics in poultry that resulted in high mortalities, mass culling, contact transmission to humans (with high fatality rates) and severe economic losses for the affected regions, with resulting trade restrictions. A significant shift in ecology of avian influenza had occurred: not only had HPAI strains adapted to enable sub-clinical infections of some highly mobile water bird species, but an HPAI reservoir had been established in the wild for the first time, from which new variants could seasonally arise and disperse as the birds migrated between their northern breeding and southern overwintering sites.

Host species differ in their innate susceptibility to avian influenza viruses, as determined by the types and distribution of cell surface receptors that the virus targets for entry, as well as the species' unique cellular immune defence capabilities. Thus, between bird orders there exists a spectrum of natural susceptibility towards infection and the ability to transmit influenza A viruses, which is also influenced by the age and immune status of the individual. On the far left of the spectrum are water birds, the natural host and reservoir. Ducks, geese and shorebirds become infected with influenza A virus but show few clinical signs, excrete virus in high quantities into the environment, but are not capable of facilitating the switch from LPAI to HPAI. In the middle, ratites such as ostriches are susceptible to infection with wild bird viruses, excrete reasonable amounts of the virus to facilitate transmission within the flock, and are capable of facilitating the mutation from LPAI to HPAI, but show few clinical signs when infected with HPAI strains. At the far right of the spectrum are gallinaceous birds such as chickens, turkeys and quail. Upon infection these birds can facilitate the mutation from LPAI to HPAI, shed great quantities of virus and are thus highly efficient transmitters, but they are highly susceptible to the disease, with lethal consequences. Where then within this spectrum are columbids positioned? Some have argued that the close association of feral pigeons with humans in urban habitats and on poultry farms, and the international movements of racing pigeons for

competition events places columbids in a high risk category for the introduction and transmission of avian influenza viruses.

The risks that columbids pose in the ecology and epidemiology of AI have been thoroughly investigated over the course of several decades. From 1985 to 2013, scientists surveyed feral and meat-type pigeons in markets in studies conducted across Asia, Europe, Africa, North America, the Caribbean and Australia, often in regions where HPAI outbreaks were ongoing at the time. Two key parameters were measured in the course these studies, namely the presence of influenza A-specific antibodies in serum which is indicative of a recent exposure to the virus, and the presence in the bird of the virus itself. In twelve studies comprising 2,046 pigeons, 8.01 % of the birds tested positive for exposure to numerous subtypes of AIV (H1Nx, H5Nx, H9Nx) based on the detection of antibodies. In 29 studies that surveyed for the presence of the virus using molecular detection assays or virus isolation, only 1.1% of the 6,155 pigeons sampled were actively shedding detectable levels of the virus. Only H3Nx, H7Nx, H9Nx and H14Nx strains were identified.

However, to directly assess the susceptibility and the ability of pigeons to transmit the virus, clinical infection studies under controlled conditions with LPAI and HPAI strains have been performed since 1944. Up until 2013, 21 international studies assessed H5N1, H5N2, H6N1, H7N1, H7N3 and H9N2 LPAI strains and H5N1, H5N2, H5N9, H7N1 and H7N7 HPAI strains *in vivo*. Out of a total 88 pigeons experimentally infected with LPAI viruses, only three birds (3.64 %) in one trial became sick or died. In this specific experiment all three pigeons were subjected to chemically-induced immunosuppression.

Similarly, in studies where pigeons were challenged with varying doses of HPAI strains, 23 out of 627 (3.67 %) of the pigeons died. Of these deaths, only 1 to 2 mortalities occurred per group, and deaths could be ascribed to concomitant infections (e.g. coccidiosis), environmental stress, or excessive viral challenge doses (exceeding 100 million egg infectious doses) that overwhelmed the birds' immune responses. Thus, more than 75 % of pigeons challenged with HPAI viruses showed no clinical signs or recovered, but where clinical signs were present, neurological signs were common. In contrast, more than 75 % of chickens challenged with the same doses and virus strains became acutely infected and died within days. Antibody detection showed that some but not all challenged pigeons seroconverted. HPAI was frequently detected in the internal organs including the brain, liver, pancreas, spleen, thymus, heart, bursa, proventriculus and intestine. Excretion levels of the virus in pigeons ranged from about 630 to 2,500 egg infectious doses from the trachea, and about 250 to 5,000 egg infectious doses from the cloaca; however, this viral shedding seemed to be below the minimum infectious dose for chickens, as no studies reported seroconversion or mortalities in chickens that were co-housed with infected pigeons. Seroconversion was detected in some contact pigeons in some studies.

The collective findings of all studies on free-living pigeons, market pigeons and those experimentally infected with LPAI and HPAI in studies from 1944 to 2013 established that, in the spectrum of susceptibility and ability to transmit the virus, pigeons are biologically at the farthest left. They do not show clinical signs when infected with HPAI viruses, are inefficient propagators and transmitters of the virus (especially to poultry), and do not facilitate the mutation from LPAI to HPAI. Cumulatively, the studies demonstrated that pigeons were of no epidemiological significance in the transmission and spread of HPAI.

The first three intercontinental transmission waves (2005 to 2015) were caused by sub-clades 2.2 or 2.3.2.1c H5N1 HPAI viruses and the pathogenicity of these strains in pigeons were assessed in several of the challenge studies outlined above. The fourth wave was caused by clade 2.3.4.4 H5Nx HPAI viruses but there was a notable shift in the viruses' ecology and epidemiology. Progressive genetic drift and multiple reassortment events with other wild bird influenza A viruses had produced clade 2.3.4.4 HPAI H5Nx viruses with different N types, e.g. H5N6, H5N2 and H5N8. Furthermore, these viruses had spread to previously unaffected regions which suggested that alternative host populations using different migration routes were acting as carriers. For the first time, the virus reached the North American continent and southern tip of the African continent. Many wild bird and domestic poultry species were affected and died following infection with the clade 2.3.4.4 H5N8 HPAI viruses, amongst which were hundreds of feral pigeons and other columbid species, usually on affected poultry farms.

Had clade 2.3.4.4 H5Nx viruses evolved to such an extent that the risk status of pigeons had changed post 2015? To assess this, a new round of pigeon challenge studies was conducted in Korea, China, the USA, South Africa and Belgium. The results of these studies were similar to those conducted prior to 2015. Chickens challenged via the intraocular or intranasal route with a standard dose of 6 million egg infectious doses of clade 2.3.4.4 HPAI H5Nx viruses excreted high quantities of virus from the trachea and cloaca before succumbing to the infection three days post challenge. In stark contrast, pigeons infected with the same doses of the viruses via the intranasal route remained clinically healthy over the trial period across all studies, with the exception of one bird with neurological signs, and excreted significantly lower numbers of viruses. Antibodies were detected in most but not all pigeons. Only one trial reported successful transmission to in-contact pigeons, without spread to co-housed chickens, confirming once again that any HPAI viruses excreted were below the minimum threshold required to establish infection in gallinaceous birds. Deaths in free-living pigeons during clade 2.3.4.4 HPAI H5Nx outbreaks were probably due to their exposure to excessive virus levels in the heavily contaminated environments of infected poultry farms, once again highlighting the importance of on-farm biosecurity and preventing access of wild birds into houses, as any wild bird can act as mechanical vectors for spread. Biologically, the longstanding status of columbids as

ineffective propagators and disseminators of HPAI and LPAI viruses prevails: the pigeon has no epidemiological significance in the maintenance and spread of avian influenza.

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